Early Evaluation of the Efficiency of Antibiotic Therapy for Nosocomial Pneumonia by Quantifying Lipopolysaccharide

A. Yu. Yakovlev[1], N. N. Gushchina[1], A. A. Niyazmatov[2], R. M. Zatsev[1], E. Yu. Golubtsova[1], M. A. Ryabikova[1]

[1] N. A. Semashko Nizhny Novgorod Regional Clinical Hospital
[2] A. N. Bakulev Research Center of Cardiovascular Surgery, Russian Academy of Medical Sciences, Moscow

Objective: **to evaluate the efficiency of intravenous and inhaled antibiotic therapy for nosocomial pneumonia caused by gram-negative bacteria by quantifying lipopolysaccharide (LPS).** ***Subjects and methods.*** **Examinations were made in 54 patients with mechanical ventilation-associated nosocomial pneumonia. Conventional de-escalating intravenous antibiotic therapy (with carbapenems) was used in Group 1 (*n*=26). Inhaled antibiotic monotherapy with TOBI (tobramycin) in a dose of 300 mg every 12 hours for 10—12 days was performed in Group 2 (*n*=28). The use of inhaled tobramycin as a monodrug was due to the absence of other infection foci. LPS was quantified using a diagnostic activated particle-method-endotox spp. kit (A. N. Bakulev Research Center of Cardiovascular Surgery, Russian Academy of Medical Sciences, OOO ROKHAT Research-and-Production Firm, Russia).** ***Results.*** **The first administration of the antibiotic is accompanied by a higher arteriovenous difference in the content of gram-negative bacterial LPS due to increased arterial endotoxemia.** ***Conclusion.*** **Elevated arterial blood LPS levels after initiation of systemic or inhaled antibiotic therapy for nosocomial pneumonia may be employed as an early criterion for its efficiency. Tobramycin inhalations give rise to a significant increase in arterial lipopolysaccharidemia as compared to de-escalation therapy with carbapenems with low rates of adverse reactions and undesirable events.** ***Key words:*** **nosocomial pneumonia, lipopolysaccharide, arteriovenous difference, inhaled tobramycin.**

Introduction

One percent of hospital patients suffer from the nosocomial pneumonia (NP) that is developed in 20% of mechanically ventilated patients. NP increases the risk of death and requires considerable treatment expenses [1—4]. The most frequent causes of NP are infections evoked by gram-negative (Pseudomonas aeruginosa, Acinetobacter spiralis., Klebsiella spiralis. etc.) and gram-positive (Staphylococcus aureus) microorganisms multiresistant to antibacterial drugs (ABD) [5]. The diversity of «problem» bacteria causing nosocomial infections and high lethality are due to inadequacy of ABD use or delay in ABD administration. These obstacles are usually circumventing by the use of evidence-based regimens of treatment with those ABD that are active against most actual germs and capable to overcome resistance mechanisms of hospital flora [6]. This is considered as a reason for a deescalation therapy [7—9].

Адрес для корреспонденции (Correspondence to):

Aleksey Yuryevich Yakovlev
E-mail: aritnnru@list.ru

Clinical and economical consequences of multiresistance of bacteria to ABD and lack of new ABD active against those pathogens stimulated the inhalationt use of ABD [10], the efficacy of which was proved in cystic fibrosis patients [11, 12]. Inhalation allows maintaining the high concentration of the medication in the infection area [13] resulting in better bacterial killing [14, 15] and lowing both systemic absorption of medication and toxicity [16].

Recently conducted pilot studies have demonstrated the efficiency of combining the systemic antibacterial therapy with inhalations. The combination therapy improved therapeutic efficacy with low toxic effects in patients [17—21]. Numerous studies had demonstrated the advantages of inhaled tobramycin versus intravenous mode in severe NP cases [22—26].

Until today the estimation of the antibacterial therapy effectiveness is based on clinical parameters (body temperature, leukocytosis, somatic status) that are available for analysis after 48—72 hours of beginning of the therapy [7, 27]. Methods of early bacterial identification in concert with the estimation of antibiotic sensitivity might have limited availability due to technological problems and high cost of analyzing equipment and operating materials. The significance of

these problems requires searching for improved criteria of primary diagnosis and evaluation of therapeutic efficacy of ABD. In this regard it seems important to study the dynamics of content of lipopolysaccharides of gram-negative bacteria in circulation as possible criteria of complications severity.

Earlier studies showed an increase in LPS binding protein and endotoxin concentrations in patients with severe pneumonia, however, the dynamics of these molecules during the antibacterial therapy was not evaluated [28, 29]. Analysis of the arterial-venous difference of gram-negative bacteria LPS level might provide information on detoxifying activities of the lungs detoxicant activity as well as on the site of the gram-negative infection in the pulmonary circuit. This suggestion has inspired the present study.

Goals of the study included determining the efficiency of antibacterial therapy based on assessment of LPS concentration in venous and arterial circulations following intravenous or inhaled ABD in patients diagnosed with nosocomial pneumonia caused by gram-negative bacteria.

Methods and materials

54 patients with NP associated with mechanical lung ventilation (MLV) were studied. In all cases NP was detected on-ste for the first time. The diagnosis was made in accordance to the requirements of Russian National Recommendations «Nosocomial Pneumonia, adults examination», 2009. X-ray scanning and computer tomography showed bilateral multisegmental character of pneumonia in all cases. Puritan-Bennet 840 devices were employed for MLV in SIMV mode with volume and pressure control. In 49 patients MLV was performed following transcutaneous tracheostomy. MLV duration prior to the study was conducted for 6—11 days. At the day of diagnosis with NP patients started to receive cephalosporins (38 patients, 70,4%) or fluroquinolones (16 patients, 29,6%) as main medication. The reason for changing the therapy was the progression of NP. According to changes the patients were subdivided in two groups. First group (26 patients) included those patients treated with the traditional deescalation therapy (intravenous injections of carbapanems). Second group of patients (28 patients) received inhalant antibiotic monotherapy (tobramycin, 300 mg every 12 hours during 10—12 days). Maximum duration of the inhaled therapy was 25 days (prescribed for a patient with the «diver trauma» due to lung destruction and high tobramycin sensitivity). Inhalations were performed with the aid of a nebulizer equipped with a bacterial filter. Inhaled tobramycin was employed as the main medication due to absence of other sites of infection and because of proved fast and equal diffusion through lung tissue regardless the initial ventilation-perfusion imbalance [30]. There were no cases of microflora resistance against the applied ABD found. Initial microbiological inoculation showed no significant difference between the groups. Patients that had gram-positive microflora in culture sputum in combination with gram-negative microorganisms were excluded from the study.

22 patients were transferred from intensive care departments of other hospitals. Groups were comparable in terms of etiology, severity of NP and comorbidity.

The results of bacteriological studies of bronchoalveolar lavage fluid harvested prior to therapy and received in 3—5 days showed domination of the multiresistant gram-negative flora and its equal distribution in both groups. Examination by disk diffusion revealed high sensitivity of the pathogenic microflora to carbapenemas and tobramycin (Table 1).

The arterial-venous difference in LPS concentrations was evaluated before the first carbapenem injection or tobramycin inhalation. It was performed with the aid of MACH-endotox spp. test (Bakoulev Center for Cardiovascular Surgery, Russian Academy of Medical Sciences, and Industrial Research Company «Rohat LLC», Russia). Then evaluation was repeated 1 hour after.

Statistical processing of the results was conducted via Microsoft Excel 2007 and StatSoft Statistica 6.0. Normality distribution of data was tested by Shapiro-Wilks test. Results showed in the study are averages with standard deviation scores. The statistical significance of differences between groups was determined at $P<0.05$.

Results and discussion

Prior to antibacterial therapy LPS level in both groups slightly exceeded control values (not more than 4 mcg/ml). LPS level in arterial blood was higher than in venous blood that can be explained by the high concentration of gram-negative bacteria and bacterial decay products in the lung tissue (Table 2). After the first intravenous administration or inhalation of ABD the LPS level in venous blood was not

Table 1

Characteristic of the patients

Criteria	Value of indicators in groups	
	1st	2nd
The average age (in years)	33,0±5,7	31,8±4,5
Male	30,8	25
Female	69,2	75
Comorbidity		
Isolated severe traumatic brain injury, %	46,2	35,7
Combined trauma, %	30,8	42,9
Brain tumor, %	11,5	3,6
Cervical spine Injury, %	11,5	17,8
Defined bacteria		
Pseudomonas aeruginosa, %	61,5	71,4
Pseudomonas maltophilia, %	11,5	—
Klebsiella pneumoniae, %	42,3	50
Acinetobacter baumannii, %	30,8	14,3
Acinetobacter calcoaceticus, %	15,4	21,4
Enterobacter alpha, %	23,1	10,7
Enterobacter cloacae, %	19,2	10,7
Serratia marcescens, %	11,5	7,1
Stenotrophomonas spp., %	3,8	10,7
Sensitivity of microflora to carbapenems and tobramycin	100	100
Score on a scale of CPIS	7,3±0,9	7,1±0,6

Table 2

Concentration of lipopolysaccharide in blood dynamics

LPS content, pg/ml	Group	Value of parameters on the stages of the research (*M*±*m*)			
		before the first application antibiotics	one hour after the first administration of antibiotics	prior to the second administrationthe of antibiotics	one hour after second administration of antibiotics
Venous blood (VB)	1st	8,5±1,4	9,0±1,5	9,2±1,1	8,9±0,9
	2nd	9,0±0,7	9,6±1,0	10,3±0,8	9,9±1,1
Arterial blood (AB)	1st	12,4±1,6**	27,6±2,0*,**	25,5±1,8*,**	26,3±2,2*,**
	2nd	13,1±1,3**	35,5±2,3*,**,#	30,6±2,0*,**,#	32,6±2,4*,**
Arterial-venous difference (AB-VB)	1st	4,1±0,4	18,4±1,7*	16,5±1,6*	17,0±1,5*
	2nd	3,8±0,4	24,8±1,9*,#	21,2±1,8*,#	23,3±1,9*,#

Note. * — $P<0.05$ vs. values from first stage of the study; # — $P<0.05$, Group 1 vs. Group 2; ** — $P<0.05$ AB vs. VB.

changed whereas endotoxin level in arterial blood increased by several times. Four patients from the first group and seven patients from the second group had 6-fold increase of endotoxemia after changing the ABD.

The same pattern remained until the next intake that kept permanently the high difference in LPS level in arterial versus venous blood. The second intake, however, did not result in additional increase of lipopolysaccharidemia. Endotoxin content in arterial blood remained considerably high during the next 5—7 days. Arterial-venous difference in LPS content had a similar timing profile with X-ray and clinical data improvements. One patient with initially low oxygenation index and excessive sputum output during the first inhalation experienced bronchospasm suppressed by broncolitic medication. In future the administration of bronchial spasmolytics and early aminophylline inhalation prevented similar complications. There was no cases of ototoxicity or nephrotoxicity when employing the inhaled tobramycin.

LPS (endotoxin, O-antigen) is a membrane structural component of all gram-negative bacteria [31]. The structural alterations of a bacterial membrane results in endotoxemia since one bacteria contain up to 3 500 000 LPS molecules. High activity of carbapenems and aminoglycosides (especially tobramycin and amikacin) against non-fermenting gram-negative flora leads to rapid killing of germs [32]. This results in a release of large number of LPS molecules into microenvironment and arterial blood stream thus providing potential benefit to employ these consequences in developing method for express evaluation of effectiveness of antibacterial therapy. This research is needed to be continued in terms of developing final recommendations and employing the latter in clinics to validate the methodology.

The fact that there was no lipopolysaccharidemia increase after the second medication intake highlights the strong antibacterial action of a prescribed first ABD.

To our knowledge, the causes of high LPS level in arterial blood remaining increased for a long time (until the regress of inflammation in the lungs), are related to the diffusion difficulties of the antibiotic inhaled or injected into foci of lung infiltrates.

The advantageous influence of tobramycin inhalations on the arterial-venous difference of LPS content can be explained by a faster germ-killig effect in sputum and bronchial/alveolar microenvironment because the intravenous injections of ABD accumulation of the active substance there is delayed due to due to alterations of drug distribution in tissues. These findings have been proved indirectly in numerous papers demonstrating clinical advantages of inhalations compared to intravenous therapy [19, 21, 33]. In our study high clinical effectiveness of the employed therapies has been demonstrated, however, any significant proof of inhalations advantages in terms of influence on titers of microorganisms in sputum has not been achieved.

Aerosol aminoglycosides therapy is carried on easily by the patients and results in high concentration of the medication in lung tissue. At the same time their serum concentration is not intense [33, 34]. This improves therapy effectiveness, its safety and decreases side effects and adverse experiences. Only few such incident have been noted in present similar studies. It is unacceptable to employ intravenous and other forms of ABD for inhalations due to common complications (bronchial and glottic spasm, coughing, damages in the mouth, gorge, trachea and bronchi, breast pain) that may defame new therapy development [35].

It is known that after the first tobramycin inhalation its concentration in lungs of cystic fibrosis patients quickly reached maximum therapeutic means that 25-fold exceeded the minimum inhibitory concentration for Pseudomonas sp. remaining at a minimum concentration in blood [36]. The same results were collected after examination of a lung cancer patient who had been receiving inhalant tobramycin before pulmonectomy [37]. This was also true for other studied antibiotics that required high concentration of ABD for effective killing of germs [38, 39]. It is believed that further improvements in treatment with ABD through employing the inhaled forms of ABD might stem from the decrease of proinflammatory cytokines produced by neutrophils and macrophages (interleukine 1β, tumor necrosis factor α etc.) as well as from the manipulating of the release of molecules of intracellular adhesion 1 (sICAM-I) reducing the neutrophilous elastase [21].

Conclusion

1. After the beginning of the systemic or inhalation antibacterial therapy in patients with NP LPS level is increased in arterial blood. This quantitative pattern seems to be an early candidate biomarker of ABD efficacy in a particular patient.

2. Inhalations with tobramycin resulted in increased arterial lipopolysaccharidemia when compared to carbapenem deescalation therapy. This treatment was rarely complicated by side reactions and adverse events.

References

1. American Thoracic Society; Infectious Diseases Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. *Am. J. Respir. Crit. Care Med.* 2005; 171 (4): 388–416. PMID: 15699079
2. *Richards M.J., Edwards J.R., Culver D.H., Gaynes R.P.* Nosocomial infections in medical ICUs in the United States. National Nosocomial Infections Surveillance System. *Crit. Care Med.* 1999; 27 (5): 887–892. PMID: 10362409
3. *Smelaya T.V., Salnikova L.E., Moroz V.V., Golubev A.M., Zarzhetsky Yu.V., Rubanovich A.V.* Geneticheskiy polimorfizm i chastota razvitiya oslozhnenii pri pnevmonii razlichnogo geneza. [Genetic polymorphism and the rate of development of complications in pneumonia of varying genesis]. *Obshchaya Reanimatologiya.* 2011; 7 (2): 10—17. [In Russ.]
4. *Khubutiya M.Sh., Shabanov A.K., Chernenkaya T.V., Godkov M.A., Dorfman A.G.* Infektsionnye legochnye oslozhneniya v reanimatsii i intensivnoi terapii u postradavshikh s sochetannoi travmoi. [Infectious pulmonary complications in intensive care unit victims with concomitant injury]. *Obshchaya Reanimatologiya.* 2011; 7 (4): 24—27. [In Russ.]
5. *Depuydt P., Myny D., Blot S.* Nosocomial pneumonia: aetiology, diagnosis and treatment. *Curr. Opin. Pulm. Med.* 2006; 12 (3): 192—197. PMID: 16582674
6. *Iregui M., Ward S., Sherman G., Fraser V.J., Kollef M.H.* Clinical importance of delays in the initiation of appropriate antibiotic treatment for ventilator-associated pneumonia. *Chest.* 2002; 122 (1): 262–268. http://dx.doi.org/10.1378/chest.122.1.262. PMID: 12114368
7. *Chuchalin A.G., Gelfand B.R. (red.).* Nozokomialnaya pnevmoniya u vzroslykh. Rossiiskie natsionalnye rekomendatsii. [Nosocomial pneumonia in adults. Russian national guidelines]. Moscow: Borges; 2009. [In Russ.]
8. *Beloborodov V.B.* Antibakterialnaya terapiya pnevmonii, svyazannoi s iskusstvennoi ventilyatsiei legkikh: put ot natsionalnykh rekomendatsii do primeneniya v otdelenii. [Antibacterial therapy for mechanical ventilation-associated pneumonia: A path from national guidelines to their use in the unit]. *Infektsii v Khirurgii.* 2011; 9 (2): 66—72. [In Russ.]
9. *Luna C.M., Aruj P., Niederman M.S., Garzón J., Violi D., Prignoni A., Ríos F., Baquero S., Gando S.; Grupo Argentino de Estudio de la Neumonıa Asociada al Respirador group.* Appropriateness and delay to initiate therapy in ventilator-associated pneumonia. *Eur. Respir. J.* 2006; 27 (1): 158–164. PMID: 16387949
10. *Wood G.C., Boucher B.A.* Aerosolized antimicrobial therapy in acutely ill patients. *Pharmacotherapy.* 2000; 20 (2): 166—181. http://dx.doi.org/10.1592/phco.20.3.166.34783. PMID: 10678295
11. *Moss R.B.* Long-term benefits of inhaled tobramycin in adolescent patients with cystic fibrosis. *Chest.* 2002; 121 (1): 55—63. http://dx.doi.org/10. 1378/chest.121.1.55. PMID: 11796432
12. *Cheer S.M., Waugh J., Noble S.* Inhaled tobramycin (TOBI): A review of its use in the management of *Pseudomonas aeruginosa* infections in patients with cystic fibrosis. *Drugs.* 2003; 63 (22): 2501—2520. http://dx.doi.org/10.2165/00003495-200363220-00015. PMID: 14609360
13. *Carcas A.J., García-Satué J.L., Zapater P., Frías-Iniesta J.* Tobramycin penetration into epithelial lining fluid of patients with pneumonia. *Clin. Pharmacol. Ther.* 1999; 65 (3): 245–250. http://dx.doi.org/10.1016/S0009-9236(99)70103-7. PMID: 10096256
14. *Goldstein I., Wallet F., Nicolas-Robin A., Ferrari F., Marquette C.-H., Rouby J.-J.* Lung deposition and efficiency of nebulized amikacin during Escherichia coli pneumonia in ventilated piglets. *Am. J. Respir. Crit. Care Med.* 2002; 166 (10): 1375–1381. PMID: 12406838
15. *Makhoul I.R., Merzbach D., Lichtig C., Berant M.* Antibiotic treatment of experimental *Pseudomonas aeruginosa* pneumonia in guinea pigs: comparison of aerosol and systemic administration. *J. Infect. Dis.* 1993; 168 (5): 1296–1299. PMID: 8228367
16. *Kahler D.A., Schowengerdt K.O., Fricker F.J., Mansfield M., Visner G.A., Faro A.* Toxic serum trough concentrations after administration of nebulized tobramycin. *Pharmacotherapy.* 2003; 23 (4): 543–545. http://dx.doi.org/10.1592/phco.23.4.543.32122. PMID: 12680485
17. *Moroz V.V., Kuzovlev A.N., Polovnikov S.G., Stets V.V., Varvarin V.V.* Ingalyatsionnyi tobramitsin v lechenii tyachelykh nozokomialnykh pnevmonii. [Inhaled tobramycin in the treatment of severe nosocomial pneumonias]. *Obshchaya Reanimatologiya.* 2012; 8 (2): 5—9. [In Russ.]
18. *Avdeyev S.N., Karchevskaya N.A., Chuchalin A.G.* Opyt ispolzovaniya ingalyatsionnogo tobramitsina pri nozokomialnoi pnevmonii. [Experience with inhaled tobramycin in nosocomial pneumonia]. *Lechebnoe Delo.* 2009; 2: 80—88. [In Russ.]
19. *Le Conte P., Potel G., Clementi E., Legras A., Villers D., Bironneau E., Cousson J., Baron D.* Administration of tobramycin aerosols in patients with nosocomial pneumonia: a preliminary study. *Presse Med.* 2000; 29 (2): 76–78. PMID: 10682031
20. *Hallal A., Cohn S.M., Namias N., Habib F., Baracco G., Manning R.J., Crookes B., Schulman C.I.* Aerosolized tobramycin in the treatment of ventilator-associated pneumonia: a pilot study. *Surg. Infect. (Larchmt).* 2007; 8 (1): 73—82. http://dx.doi.org/10.1089/sur.2006.051. PMID: 17381399
21. *Ghannam D.E., Rodriguez G.H., Raad I.I., Safdar A.* Inhaled aminoglycosides in cancer patients with ventilator-associated Gram-negative bacterial pneumonia: safety and feasibility in the era of escalating drug resistance. *Eur. J. Clin. Microbiol. Infect. Dis.* 2009; 28 (3): 253—259. http://dx.doi.org/10.1007/s10096-008-0620-5. PMID: 18752007
22. *Mohr A.M., Sifri Z.C., Horng H.S., Sadek R., Savetamal A., Hauser C.J., Livingston D.H.* Use of aerosolized aminoglycosides in the treatment of gram-negative ventilator-associated pneumonia. *Surg. Infect. (Larchmt).* 2007; 8 (3): 349—357. http://dx.doi.org/10.1089/sur.2006.041. PMID: 17635058
23. *McCall C.Y., Spruill W.J., Wade W.E.* The use of aerosolized tobramycin in the treatment of a resistant pseudomonal pneumonia. *Ther. Drug Monit.* 1989; 11 (6): 692–695. PMID: 2595751
24. *Chastre J., Wolff M., Fagon J.Y., Chevret S., Thomas F., Wermert D., Clementi E., Gonzalez J., Jusserand D., Asfar P., Perrin D., Fieux F., Aubas S.; PneumA Trial Group.* Comparison of 8 vs 15 days of antibiotic therapy for ventilator-associated pneumonia in adults. *JAMA.* 2003; 290 (19): 2588–2598. http://dx.doi.org/10.1001/jama.290.19.2588. PMID: 14625336
25. *Beloborodov V.B.* Prakticheskie rekomendatsii po diagnostike i lecheniyu nozokomialnoi pnevmonii: chto novogo? [Practical guidelines for the diagnosis and treatment of nosocomial pneumonia: What is new?] *Infektsii i Antimikrobnaya Terapiya.* 2005; 2: 60—66. [In Russ.]
26. *Khairullina R.M., Mavzyutov A.R., Fazlyeva R.M., Akbasheva A.O., Kuzovkina O.Z.* Sostoyanie antiendotoksinovoi zashchity pri vnebolnichnoi pnevmonii. [The antiendotoxin defense in community-acquired pneumonia]. *Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii.* 2010; 4: 65—71. [In Russ.]
27. *Matkevich V.A., Luzhnikov E.A., Ilyashenko K.K., Petrov S.N., Nikulina V.P., Evdokimova N.V.* Vliyanie kishechnogo lavazha na razvitie pnevmonii u bolnykh s ostrymi otravleniyami psikhofarmakologicheskimi sredstvami. [Impact of intestinal lavage on the development of pneumonia in patients with acute poisonings by psychopharmacological agents]. *Obshchaya Reanimatologiya.* 2011; 7 (2): 20—24. [In Russ.]
28. *Grotta M.B., Etchebere E.C., Ribeiro A.F., Romanato J., Ribeiro M.A., Ribeiro J.D.* Pulmonary deposition of inhaled tobramycin prior to and after respiratory therapy and use of inhaled albuterol in cystic fibrosis patients colonized with *Pseudomonas aeruginosa*. *J. Bras. Pneumol.* 2009; 35 (1): 35—43. PMID: 19219329
29. *Mayansky A.N.* Mikrobiologiya dlya vrachei. [Microbiology for physicians]. Nizhny Novgorod: NGMA; 1999: 127. [In Russ.]
30. *Yakovlev V.P., Yakovlev S.V., Aleksandrova I.A.* Ratsionalnaya antimikrobnaya terapiya. [Rational antimicrobial therapy]. Moscow: Litterra; 2007: 783. [In Russ.]
31. *Palmer L.B., Smaldone G.C., Simon S.R., O'Riordan T.G., Cuccia A.* Aerosolized antibiotics in mechanically ventilated patients: delivery and response. *Crit. Care Med.* 1998; 26 (1): 31–39. http://dx.doi.org/10.1097/00003246-199801000-00013. PMID: 9428540
32. *Goldstein I., Chastre J., Rouby J.J.* Novel and innovative strategies to treat ventilator-associated pneumonia: optimizing the duration of therapy and nebulizing antimicrobial agents. *Semin. Respir. Crit. Care Med.* 2006; 27 (1): 82–91. http://dx.doi.org/10.1055/s-2006-933676. PMID: 16508884
33. *Conrad D.J.* The clinical use of aerosolized antibiotics. *Clin. Pulm. Med.* 2003; 10 (4): 201—220. http://dx.doi.org/10.1097/01.cpm.0000080903.11193.e9.
34. *Geller D.E., Pitlick W.H., Nardella P.A., Tracewell W.G., Ramsey B.W.* Pharmacokinetics and bioavailability of aerosolized tobramycin in cystic fibrosis. *Chest.* 2002; 122 (1): 219–226. http://dx.doi.org/10.1378/chest.122.1.219. PMID: 12114362
35. *Le Conte P., Potel G., Peltier P., Horeau D., Caillon J., Juvin M.E., Kerguéris M.F., Bugnon D., Baron D.* Lung distribution and pharmacokinetics of aerosolized tobramycin. *Am. Rev. Respir. Dis.* 1993; 147 (5): 1279—1282. http://dx.doi.org/10.1164/ajrccm/147.5.1279. PMID: 8484643
36. *Ramsey B.W., Pepe M.S., Quan J.M., Otto K.L., Montgomery A.B., Williams-Warren J., Vasiljev K.M., Borowitz D., Bowman C.M., Marshall B.C., Marshall S., Smith A.L.* Intermittent administration of inhaled tobramycin in patients with cystic fibrosis. *N. Engl. J. Med.* 1999; 340 (1): 23–30. http://dx.doi.org/10.1056/NEJM199901073400104. PMID: 9878641
37. *Touw D.J., Jacobs F.A., Brimicombe R.W., Heijerman H.G., Bakker W., Briemer D.D.* Pharmacokinetics of aerosolized tobramycin in adult patients with cystic fibrosis. *Antimicrob. Agents Chemother.* 1997; 41 (1): 184–187. PMID: 8980777

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